DEVELOPMENT OF RAPID METHODS FOR THE ESTIMATION OF THE OIL CONTENT OF SINGLE COTTONSEEDS

Part III.—Critical Examination of the Oil-expression Method

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The method utilizing the expression of oil between several discs of filter paper has been exhaustively tested, and a detailed assessment of the conditions governing its accurate utilization is made. The law connecting the area of the oil spot with crushing load is obtained and a technique of correcting for the varying water content of the kernel is worked out. The influence of the wetness of the filter discs has been separately determined, and measurements at an optimum relative humidity of about 80% are recommended.

While the diameter and grade (i.e. coarseness or fineness) of the filter paper are shown to have a very small effect, of the order of ± 3%, on the results, the size of the oil spot is found to be inversely proportional to weight per unit area of the discs, as was anticipated from theory. This makes it possible to directly correlate measurements made with different types of filter papers. The most favourable conditions for reproducibility and accuracy are noted, and a constant is obtained for direct conversion of area of oil spot into milligrams of oil present in the kernel.

1. Introduction

The rapid estimation of oil content on small samples of cottonseeds is a matter of considerable importance for the selection of suitable batches of seeds for propagation in experiments on the breeding of high-oil-content varieties. The smallest possible sample appears to be a single cottonseed, and in an earlier communication,[1] experimental results were presented to show the feasibility of determining the oil content of a single cottonseed by expression of the oil between several discs of filter paper.

The kernel from the dehulled cottonseed is placed between several discs of filter paper, an equal number on either side, and the sandwich is then placed between the plattens of an ordinary 10-ton laboratory hydraulic press. A suitable crushing load is applied, and on removal from the press, the sandwich presents the appearance shown diagrammatically in Fig. 1 (top). The dark patch in the centre is the crushed non-oily portion of the kernel and surrounding this is the slightly elliptical spot made by the expelled oil, the edges of which spot are very sharp, allowing accurate measurement of its diameters to 0.1 mm. with a low-power microscope or by estimation with a millimetre scale. The mean of two mutually perpendicular diameters leads to an exact value of the total area of the spot. Errors of measurement are further reduced by repeating the measurements on several of the filter papers forming the sandwich. With six filter dics, the area of the oil spot is of the order of 200 sq. mm. for the average cottonseed.

This method has now been tested exhaustively and in the present paper, a detailed assessment of the conditions governing its accurate utilization is made, and a value is obtained for the conversion factor connecting the oil content with the effective area of the oil spot.

2. Law connecting the Crushing Load with the Area of the Oil Spot

In the experiments described in the previous communication,[1] cottonseeds of the L. S. S. variety were mostly used, and it was found (Fig. 1 (bottom)) that, in the range of 5 to 10 tons crushing load, a maximum sensitivity, defined as area of

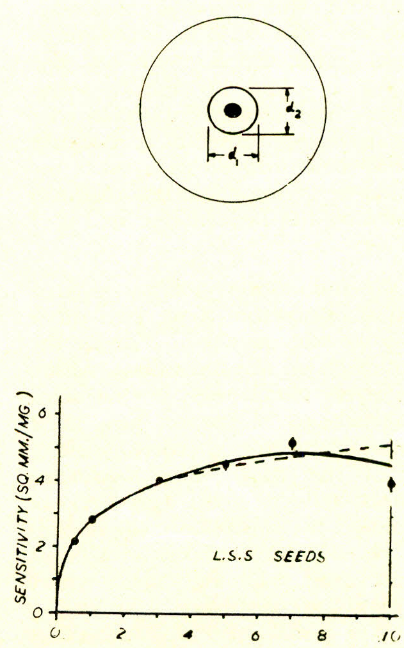

Fig. 1. (Top) Diagramatic sketch of the filter discs with crushed kernel and oil spot. (Bottom) Previously reported plot of sensitivity (i.e. area of oil spot per mg. of kernel) against load, obtained with L. S. S. Cottonseeds.

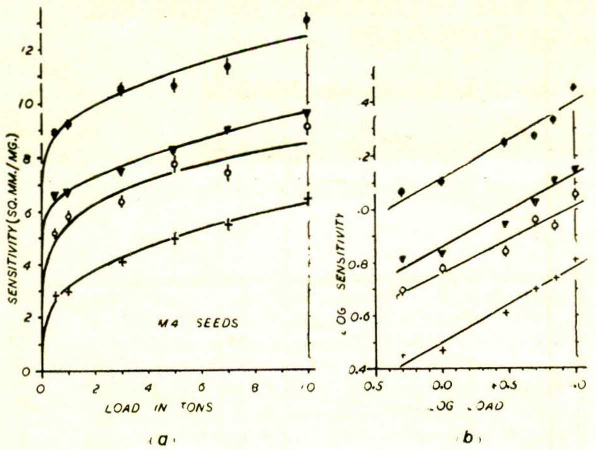

Fig. 2.—Plots of measurements with several lots of M4 variety of cottonseeds for determining the law for the sensitivity-load curve: (a) sensitivity against load and (b) logarithm of the sensitivity against logarithm of the load, the latter yielding straight lines in every case.

oil spot per mg. of kernel, was obtained. Since there are indications of unusually large variations in the percentage oil content of seeds of the L. S. S. variety,[2] it was considered desirable first to check the previous measurements by working with another variety, namely M 4. Typical sets of readings obtained with several lots of (24) M 4 cottonseeds under varying conditions (at different times of the year) are shown in Fig. 2(a), and they all indicate a slow monotonic increase in area of oil spot with increasing load, and this feature has been confirmed in all the other varieties examined. The precise reason for the shallow maximum previously observed with L.S.S. seeds has not been ascertained, but it could be due to some unduly large, positive and negative variations in the readings at 7 and 10 tons, respectively, because a curve in reasonable concordance with those for M 4 seeds is obtained by giving these last readings less weights as shown by the broken line in Fig. 1 (bottom).

At all events, the monotonic increase in area with crushing load is reasonable if we remember that a fixed volume of the oil expelled from the kernel has been absorbed on the filter discs, which are now being uniformly compressed by the load. The reduction in thickness of the oil film must therefore be compensated by a corresponding increase in area of the spot. The analytical form of the relation between load and area of spot can be determined by plotting the logarithm of the sensitivity i.e. area/mg. of kernel against the logarithm of the load, as in Fig. 2(b), where successive sets of points are displaced upwards by 0.2 unit. The sets of points give approximately linear and parallel plots, whose equations can all be represented by

$$\log S = A + B \log (\text{Load}/10), \quad (1)$$

where B lies between 0.25 and 0.29 and A (i.e. $\log S_{10 \text{ tons}}$) varies from 0.73 to 0.81, the mean values being 0.27 ± 0.01 and 0.78 ± 0.03, respectively. Thus, from equation (1) we can get

$$S = 10^{0.78} \times (\text{Load}/10)^{0.27}$$
$$= 6.0 \times (\text{Load}/10)^{0.27} \quad (2)$$

as the mean equation of the sensitivity curves of Fig. 2(a).

The variation in the value of A for the individual graphs of Fig. 2(b) is considerably greater than might reasonably be expected from the estimated standard errors of the experimental points, and further examination of the data showed that the lowest value (0.73) corresponds to the data (triangles) obtained under a prevailing relative humidity of about 45%, while the highest value (0.81) is obtained from the experiment (hollow circles) carried out at a time of the year when the relative humidity was nearly 85%. This correlation suggested a more detailed study of the influence of atmospheric humidity on the area of the oil spot obtained at different loads. This influence can be two-fold, viz.

(a) increase in area of oil spot due to increased water content of the kernel,

(b) variation produced by a change in the compressibility and porosity of the filter paper on exposure to high humidities,

and those two effects are investigated separately in the following.

3. Influence of the Water Content of the Kernel and Postulation of "Effective Area of Oil Spot"

For this investigation, an auxiliary experiment was first conducted to determine the relationship between the relative humidity and the water-content of the kernels used (M 4 variety of cottonseed). Several humidifiers, each giving a specified relative humidity, were made by placing saturated solutions of various salts (in place of the usual desiccant) in a series of desiccators. Two sets of three kernels of M 4 cottonseeds were then run through one complete hydration $\rightleftharpoons$ dehydration cycle, starting with decreasing humidity for one set and increasing humidity for the other, and the weight of each kernel was recorded at each of the five humidities available. The mean results obtained as weight % water are plotted in Fig. 3, together with the corresponding data for the cottonseed cake (broken line) from seeds of the M 4 variety, the vertical lines through the plotted

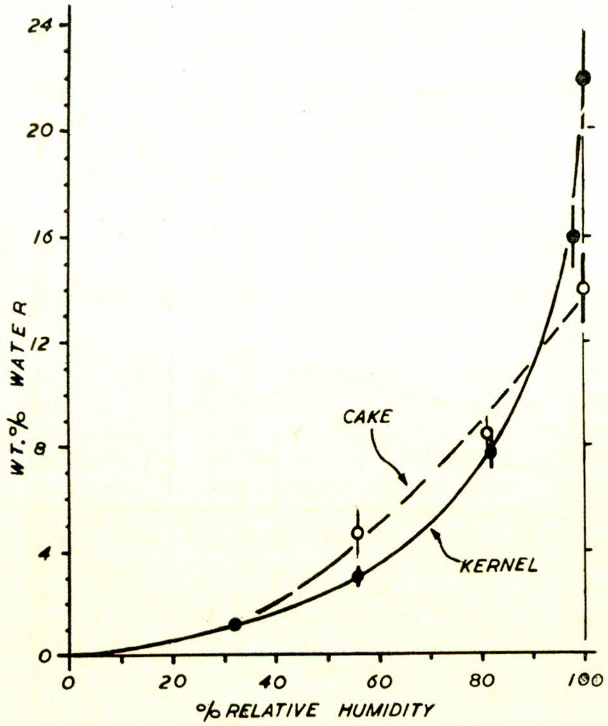

Fig. 3.—Graphs showing the measured variation of water content of cottonseed kernel (solid cirlces, full line) and cake (hollow circles, broken line), plotted against percent relative humidity at an ambient temperature of 26°C. The steep rise in the right half of the graph is to be noted.

points indicating the experimentally estimated standard errors. It is seen that up to a relative humidity of 70%, the water-content of the kernels increases rather slowly, and thereafter rises rapidly to over 20% at 100% humidity. On the basis of this graph, a suitable distribution of humidities was selected for the main experiment, and values of 60% and 90% were fixed upon between the extreme values of 0% and 100%, and four desiccators were arranged to give nearly these four values of humidity. The actual values of the humidity obtained were 0%, 56 ± 1%, 89 ± 1% and 100%. In addition, some readings were taken using the atmospheric humidity of 85 ± 2%.

Five lots of 24 kernels of the M 4 variety of cottonseeds were placed in these five humidities, and the kernels were removed one by one after a period of 30 hours, which sufficed for the attainment of equilibrium. Each kernel was weighed and then placed in a sandwich of six filter papers (Greens 401, dia. = 11 cm.) and crushed at the appropriate load, after which the mean diameters of the oil spot as well as the crushed kernel were measured to 0.1 mm. The room temperature at this time was 26° to 28°C. and the atmospheric

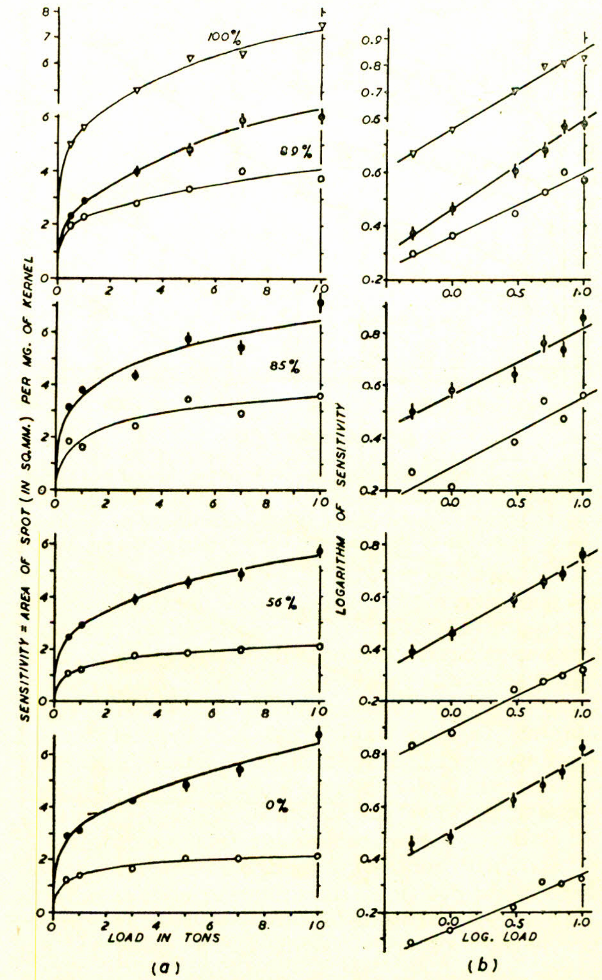

Fig. 4.—(a) Normal plots and (b) logarithmic plots for sensitivity against load, obtained with M4 cottonseed kernels maintained at several different relative humidities (noted as % in the figure), the filter papers being kept at the ambient humidity of 85% ± 2%, Solid circles for area of oil spot, hollow circles for area of crushed kernel (triangles at 100% relative humidity).

relative humidity was 85 ± 2%. The results of these measurements are plotted on Fig. 4(a) in terms of the areas of oil spot and of crushed kernel per mg. of kernel, the abcissae being the crushing loads used. Fig. 4(b) shows the corresponding logarithmic plots.

All the curves have essentially the same shape, and can be fitted by equations of the type log S=A+B log (Load/10). We may therefore use the value of S at a suitably selected load, say 7 tons, for a detailed analysis of the effect of humidity. In Fig. 5 (top half) are shown the values of S_7 for the oil spot (hollow circles) and of the corresponding quantity for the area of crushed kernel (S_7, crosses) against relative humidity and water content of

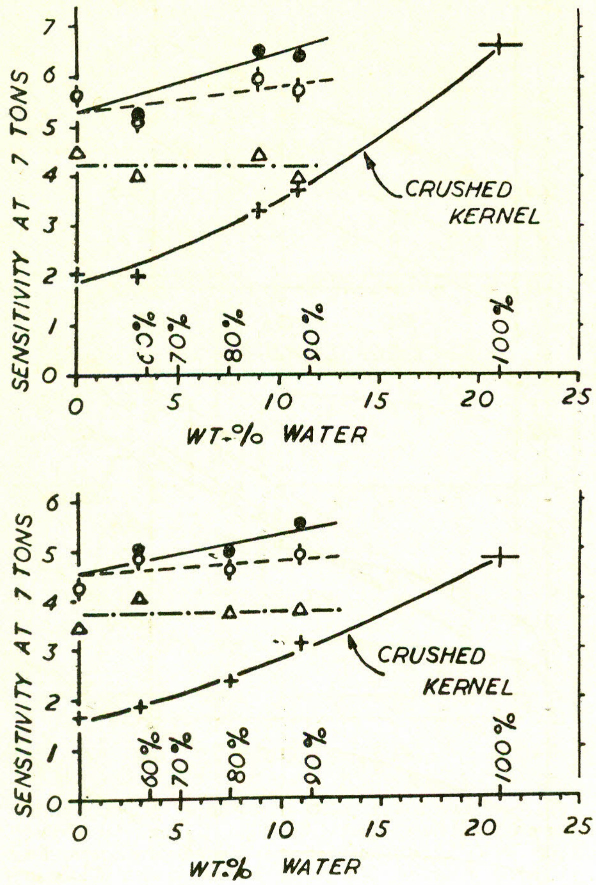

Fig. 5.—Plots against wt.% water (in kernel) for the sensitivity at 7 tons: (i) hollow circles, on the basis of wet weight of kernels (ii) solid circles, on the basis of dry weight of kernels (iii) crosses, for the area of crushed kernels only (iv) hollow triangles, for the "Effective" area of oil spot, also on the basis of dry weight.

The upper half of the figure gives the results obtained from Fig. 4 with the filter paper at 85% relative humidity, while the lower half gives those based on Fig. 6 with the paper at 40% relative humidity.

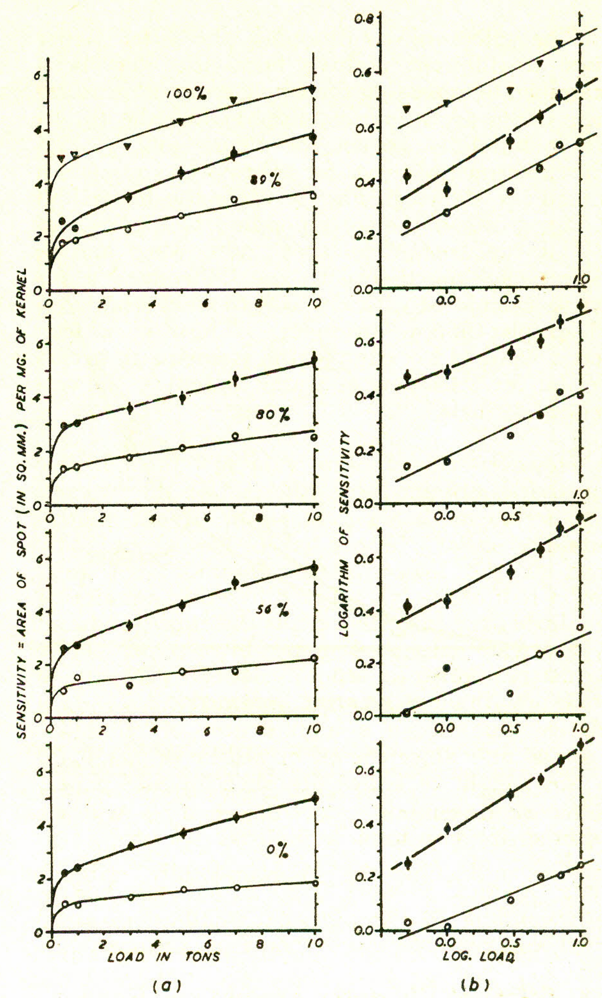

Fig. 6.—Plots as for Fig. 4, but with filter papers maintained at 40± 2% relative humidity.

the kernel. Whereas the five points for area of crushed kernel show a marked progressive increase with increasing water content, those for the area of the oil spot show a relatively smaller percentage change, at least from 0% to 89% relative humidity i.e. 0% to 11% water content. (With the fully saturated kernels, the oil spot could not be observed because of the large and uneven spread of the soft kernel.) This near constancy is however fortuitous, because the weights of the kernels include the varying weights of water absorbed under the several degrees of humidity, and when the areas of oil spot/mg. of kernel are reduced to the basis of a standard state, e.g. complete dryness (or 10% moisture content), they are found to increase substantially with water content of the kernel (Fig. 5 (top half), solid circles). This increase is, however, about 0.6 of the corresponding increase in the area of the crushed kernel.

It follows that if we define an "Effective Area of the Oil Spot" by the relation

$$A_{eff} = (A_{total} - \alpha \times A_{kernel}) \quad (3)$$

and calculate the effective sensitivity on the basis of A_{eff} instead of A_{total}, then the effective sensitivity will be nearly independent of the water content of the kernel if $\alpha = 0.6$. The corresponding effective sensitivities are plotted as triangles in Fig. 5 (top half) and are seen to yield a horizontal graph within the limits of the experimental errors. The above result can be interpreted physically by assuming that only about half of the water in the kernel is expelled on to the filter paper, while the rest remains bound in the crushed kernel.

4. Influence of Wetness of Filter Paper

The effect of conditioning the filter paper discs at different realtive humidities has been examined by repeating the whole series of measurement under dry winter conditions when the atmospheric humidity lay in the range 40% ± 2%. The experimental curves for sensitivity as a function of load are shown in Fig. 6, while the lower half of Fig. 5 shows the corresponding plots of S_7 and S'_7 for the area of oil spot and crushed kernel, respectively, together with the "effective" sensitivities calculated with $\alpha = 0.5$ to yield a horizontal graph (hollow triangles). Thus, it is seen that a value of $\alpha = 0.55 \pm 0.05$ is adequate to correct for the variation in water content of the cottonseed kernels.

However, the absolute values of the effective sensitivities obtained are different for the two sets of experiments, being 4.15 at the higher relative humidity of 85% (upper half of Fig. 5) and only 3.72 at the lower relative humidity of 40% (lower half of Fig. 5). The difference, amounting to a little over 10%, represents the effect produced by the water absorbed in the filter paper at the higher humidity, and points to the necessity of working with filter papers maintained at a standard relative humidity if a high degree of reliability is aimed at. Since the effect will be proportional to the weight of water absorbed by the paper, rather rigid humidity control (± 2%) will be required if very high humidities (~ 90%) are employed, cf. the graph of Fig. 3. However, it seems practical to work at 80% relative humidity corresponding to the accepted standard of 10% water content for measurements on cottonseeds.

5. Effect of Diameter, Weight and Grade of Filter Paper

There remain for consideration the effects of the diameter, weight and grade of filter paper employed in this method of oil estimation. From a given packet of 11 cm. diameter filter papers, several sets of discs 5.5 cm. dia. and 3.5 cm. dia. were cut out, and measurements of the oil spot at various loads were made with these small filter discs, following the usual procedure and using a fixed relative humidity of 60%. The resulting graphs for log sensitivity against log Load are shown in the lower half of Fig. 7, and it is at once apparent that the sensitivity increases as the discs are made smaller. However, the influence of size of disc is small in the range of 11 cm. to 5.5 cm. diameter, amounting to less than 0.02 in log S_7 i.e. less than 5%. The curve for the 3.5 cm. dia. disc is uniformly higher than the others by about 0.07, corresponding to a factor of about 1.2. Thus, discs of diameter from 5 cm. to 11 cm. will be quite suitable for obtaining accurately reproducible results.

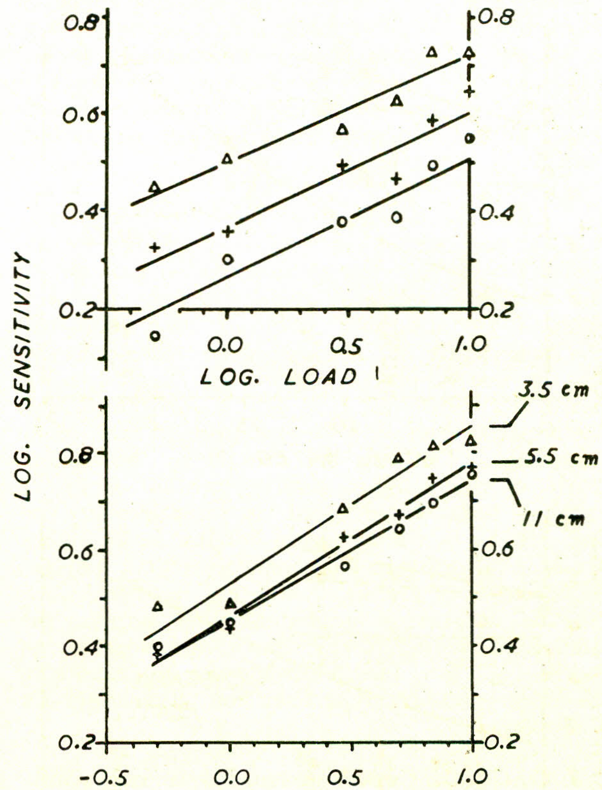

Fig. 7. (Lower half) Logarithmic sensitivity graphs obtained with three different sizes of filter discs, 11 cm., 5.5 cm., and 3.5 cm. dia. (Top half) Logarithmic "effective" sensitivity graphs for three different grades of filter papers hollow circles, Whatman No. 41; crosses, Whatman No. 1; triangles, Whatman No. 42. Successive graphs have been shifted upwards by 0.1 unit for clarity.

Similarly, the effect of changing the grade i.e. the texture of the filter paper was examined by obtaining logarithmic "effective" sensitivity curves (using effective area of oil spot) under constant conditions (R. H. = 81%), but using three different grades of 11 cm. diameter paper, viz. (1) Coarse (Whatman 41, wt. = 796 ± 9 mg.), (2) Medium (Whatman 1, wt. = 777 ± 8 mg.), and (3) Fine (Whatman 42, wt. = 821 ± 6 mg.). These graphs are shown in the upper half of Fig. 7, the successive plots being shifted upwards by 0.1 unit to prevent confusion due to overlapping. Although the scatter of the points is considerable because only 2 to 3 seeds were used at each load, it can be seen that the values of log S_7 for the three grades lie between 0.46_2 (medium) and 0.485 (fine), and represent a mean of 0.47_2 with a variation of ± 0.010, which corresponds to

$\pm$ 2.5% in S_7. Therefore the effect of changing the grade of the filter paper is only of the order of 2 to 3%. This result is not substantially altered when account is taken of the varying weights of the filter papers, by dividing the above sensitivities by the respective areas for unit weight, see below.

Variation in weight of the filter paper will clearly produce a corresponding change in the sensitivity because the area of the oil spot should be inversely proportional to the mass per unit area of the absorbing material. This effect can be seen clearly by comparing the mean effective S_7 for the Whatman papers, viz. 2.96 $\pm$ 0.05, with the figure of 4.10 $\pm$ 0.5 for the Greens 401 paper (wt. = 568 $\pm$ 2 mg.) at the same humidity. The two are exactly in the inverse ratio of the weights, and a constant figure of 2.35 $\pm$ 0.01 is obtained by multiplying the effective S_7 by the weight of one disc in grammes. It is noteworthy in this connection that, whereas a variation of as much as $\pm$ 4% has been noted in the weights of individual filter papers, the means of five (as given above) are constant to within $\pm$ 1%. This points up the importance of using a sandwich of at least four filter discs for one kernel.

6. Optimum Conditions and Determination of Calibration Constant by Comparison with Soxhlet Extraction

On the basis of the foregoing results, we can fix upon the following as optimum conditions for the use of this method of oil estimation, viz.

(i) Diameter of filter discs to be 5 to 11 cm.
(ii) Filter discs to be maintained at a controlled relative humidity, preferably about 80%.
(iii) A sandwich of at least 4 discs to be used for one kernel.
(iv) Crushing Load to be about 5 to 10 tons and "Effective" area of oil spot to be used to eliminate effect of water content of kernel.
(v) The mean weight of the sandwich of filter discs should be recorded if the highest precision is aimed at.

The final step was to determine a constant factor for direct conversion of the area of oil spot measured as above into the oil content of the kernel. This has been done by taking a sample of about 20 g. cottonseeds of a given variety and dividing it into four representative sub-samples by successive "quartering". One of these four sub-samples was used for measuring the mean area of oil spot per mg. of kernel, while another was used for making three determinations of oil content (after dehulling) by the usual soxhlet method. (About 5 to 10% of the kernels were found to be partly shrivelled). The results obtained with cottonseeds of the M 4 variety, using sandwiches of four Green's 401 filter papers (5.5 cm. dia, R. H. = 81%), are given below:

Effective Sensitivity = 5.10$\pm$0.15 sq. mm./m.g of kernel (on 10% moisture basis)

Measured oil content = 27.5% $\pm$ 1.0% of kernel (on 10% moisture basis),

that is = 0.275 $\pm$ 0.010 mg. per mg. of kernel

$\therefore$ Conversion factor

$$= \frac{\text{Measured oil content per mg. kernel}}{\text{Effective Sensitivity}}$$

$$= \frac{0.275 \pm 0.010}{5.10 \pm 0.15} = 0.054 \pm 0.002 \text{ mg./sq. mm.}$$

Similar determinations were made with a sample of L. S. S. cottonseeds and gave the value

$$\text{Conversion factor} = \frac{0.328 \pm 0.015}{5.60 \pm 0.12}$$

$$= 0.058 \pm 0.002 \text{ mg./sq. mm.},$$

thus yielding a mean factor of 0.056 $\pm$ 0.002 mg./sq. mm. for conversion of the "effective" area of oil-spot into the quantity of oil.

It is satisfying to note that, while the errors in the above determination are of the order of 5%, the two values for the constant obtained with two different varieties of cottonseeds are in good agreement with each other, so that the method can now be applied to other varieties with a satisfactory measure of confidence. Also, the reliability of 2 part in 50 for the above mean value of the constant implies that oil content determination on a kernel containing about 10 mg. of oil can be made to within 0.4 mg. i.e. to about $\pm$ 0.4% on the weight of the delinted seed. This degree of accuracy appears to be satisfactory for most purposes.

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